# MAST CELL POPULATION IN HUMAN SKIN*

GEORGE R. MIKHAIL, M.B., B.Ch., D.D.V., M.S. AND ANTONINA MILLER-MILINSKA, M.D.

The morphology and physiology of the mast cell have been the subject of extensive studies by numerous investigators (1, 2, 3, 4). However, insufficient data exist in the literature concerning the mast cell population of the skin. Since the reports dealing with this aspect of cutaneous histology have been conducted, with one exception (22), on cadavers or have been based on personal impressions, the authors felt it would be appropriate to accumulate figures concerning the number of mast cells in relation to age, race, and sex, and on their distribution according to regions of the body, in an attempt to find a standard by which variations from the normal may be detected and which may serve as an aid in the study of skin diseases.

## MATERIAL AND METHODS

Seven healthy adult volunteers free from skin or systemic diseases were selected for the study. Punch biopsy specimens were obtained from each subject from at least four of six different body areas. The sites studied were the forearm, the arm, the anterior chest wall, the back, and the extensor surfaces of the thigh and the leg. In all, 34 specimens were examined in this group (Table I). Counts were also made in 33 skin biopsy specimens which had been taken from 33 patients suffering from various conditions but were found histologically free from pathological changes (Table II). In addition, the mast cell population was determined in 17 dermatological conditions occurring in 28 patients (Table III). In four patients belonging to the last group normal skin material was also examined (Table III).

In the seven control volunteers, the 34 skin specimens were obtained under local infiltration anesthesia with 1% xylocaine solution without epinephrine. All these specimens were fixed in absolute ethyl alcohol. Almost all of the other 62 tissues included in the study were fixed in 10% formalin solution.

The tissues were imbedded in paraffin and sectioned at 25 micra. The sections were stained according to the method described by Montagna *et al* (5), with some modification. Toluidine blue was used in a strength of 0.025%, and the tissues were stained in it for 18 hours after buffering to pH 3, 4 and 5. The sections were then rinsed in distilled water, placed 2–3 minutes each in 2 changes of tertiary butyl alcohol, and 2 minutes in absolute ethyl alcohol, and finally cleared in xylene and mounted.

Counting was undertaken by means of an ocular net micrometer disc, and the tissue area covered by it was ascertained by means of a Neubauer counting chamber. The thickness of each section was also determined by the microscopic stage micrometer. The thickness varied from 23 to 26 micra, with a mean value of 24.7 micra. Therefore, in the final calculations all sections were considered to be 25 micra thick. In order to obtain values representing actual cell distribution, care was taken, during the counting, to study contiguous fields. Furthermore, each field was counted independently by each of the authors and the results compared. Any cell exhibiting metachromasia was identified as a mast cell.

## RESULTS

The fine and densely packed reddish purple metachromatic mast cell granules were most prominent when the tissue was fixed in absolute alcohol and stained in toluidine blue solution at pH 3. Sections prepared and stained by this method render the task of counting an easy one. This is demonstrated by the fact that the counts made by both authors were generally identical.

Mast cells were found most numerous in the vicinity of blood vessels and around hair follicles. On rare occasions mast cells were seen in the epidermis (Fig. 1). Morphologically, the mast cell takes various shapes and different sizes, which may occur in the same preparation. Round, oval, oblong, spindle-shaped and dendritic forms were observed in the cases studied (Fig. 2). Mast cells adjoining the epidermis, and those seen in the epidermis among the keratinocytes, were conspicuously smaller in size and poorer in granules than the mast cells of the deeper corium (Figs. 1, 2). Also, the nuclei of the superficially lying mast cells stained darker than those of the more deeply situated cells.

In the control group consisting of seven adults 27–50 years of age, the mast cell population per cubic millimeter varied between 5,120 and 9,472, with a mean value of 7,225. No statistically significant differences could be found with relation to the site of origin of the biopsy preparation, or on the basis of sex or racial differences (Table I).

The 33 biopsy preparations not exhibiting abnormalities had been taken from patients suf-

* From the Department of Dermatology, Wayne State University College of Medicine and Detroit Receiving Hospital, Detroit, Michigan.

Supported in part by grants from the United States Public Health Service through the National Institutes of Health No. AM-07194 and 2A-5267 and by a research contract from the U.S. Army Research and Development Division DA 49-007-MD-584.

Received for publication December 30, 1963.

TABLE I

*Mast cell counts in 34 biopsy specimens from six different skin sites in seven healthy volunteers*

| Subject | Age | Sex | Race | Mast Cells per Cubic Millimeter of Corium | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | Arm | Forearm | Chest | Back | Thigh | Leg |
| #61 | 40 | F | W | 6,528 | 6,784 | 6,272 | — | 7,296 | 8,064 |
| #63 | 50 | F | W | 7,424 | 9,344 | — | 6,528 | 6,528 | 6,272 |
| #62 | 48 | M | W | 6,912 | — | 9,088 | — | 7,272 | 7,296 |
| #64 | 27 | M | W | 7,424 | 5,120 | 7,168 | 9,472 | 6,400 | 6,144 |
| #66 | 39 | M | C | 6,656 | — | 7,552 | 7,936 | 6,400 | 8,320 |
| #67 | 37 | M | C | 6,912 | — | 7,294 | 7,166 | 7,936 | — |
| #68 | 38 | M | C | 7,294 | — | 6,144 | 7,422 | 8,448 | 6,656 |
| Mean for each area: | | | | 7,021 | 7,082 | 7,236 | 7,704 | 7,182 | 7,125 |
| Mean for all areas: | | | | 7,225 | | | | | |

fering from some disorder. The operations were undertaken in most instances under xylocaine-epinephrine infiltration anesthesia, and the specimens were fixed in 10% formalin solution. The great discrepancy in the mast cell counts in such cases, from a lowest of 1,280 to 10,112 per cubic millimeter (Table II), might have been due to one or more of the following factors: general disease or hormonal influences, degranulating effect of epinephrine in the local anesthetic, drug or medication effects, inadequate fixation of the mast cell granules. Although these same influences may have affected the counts obtained in 29 biopsy specimens from 17 dermatological conditions (Table III), some relevant information may become apparent if one considers the normal mast cell population to be within the 5,120-9,472 range or its whereabouts. It may be noted that, apart from urticaria pigmentosa, high counts were obtained in pretibial myxedema, Paget's disease of the nipple, atopic dermatitis, mycosis fungoides, neurofibromatosis, erythroderma (chronic), hypertrophic lichen simplex chronicus and lichen planus. On the other hand, the mast cell count seemed to be diminished in cases of acute eczematous dermatitis, seborrheic dermatitis and sarcoidosis. Although the count in the case of squamous cell carcinoma falls within the normal range, one has to bear in mind that mast cells exist in the stroma of the tumor only and, therefore, since contiguous microscopic fields were always counted, the figure of 7,680 is really a high one. The counts obtained in discoid lupus erythematosus, psoriasis, chronic contact dermatitis, and keratosis senilis, seem to be within the normal range.

## DISCUSSION

The population of mast cells in tissues is influenced by many factors. Mastocytogenesis is stimulated by the somatotropic hormone of the anterior pituitary and estrogen, whereas it is inhibited by epinephrine, thyroxine, cortisone and the adrenocorticotropic hormone of the anterior pituitary (4, 6, 7, 8, 9). Of the drugs which decrease the mast cells or have a degranulating effect upon them may be mentioned morphine (3), polymixin B, reserpine (10), acetylsalicylic acid (11), and compound 48/80 (12).

Brack (13) and Hellström and Holmgren (14) found that the skin of children contained a much higher proportion of tissue mast cells than the skin of adults. However, the mast cell rate seems to drop more rapidly with age in women than it does in men (14). Williams (15) and Sundburg (16), on the other hand, found that tissue mast cells became more numerous with advancing age. In Sundburg's study the mast cell population in the tunica adventitia of the abdominal aorta, and the subclavian and the external iliac veins was determined in autopsy material. The average rates in the abdominal aorta in three twenty-year age groups, 40–59 years, 60–79 years, and over 80 years, were 1292, 1542 and 1564 cells per cubic millimeter, respectively. The same pattern of increase with age was observed in the subclavian and external iliac veins.

During acute inflammatory conditions the tissue mast cells decrease in number and may temporarily disappear (2, 3). For some unknown reason the cells undergo rapid disintegration, and the freed granules are phagocytized by neighboring fibroblasts (15, 17). Later, as the acute process subsides, there occurs a progressive increase in the number of mast cells in the affected area. Finally, in sclerotic scar tissue the local mast cell population is characteristically nil. In contrast to acute inflammation, the chronic variety is always accompanied by an increase of mast cells. Such increase has been observed in many skin diseases. Examples which may be cited are urticaria pigmentosa, neurodermatitis, lichen planus, urticaria, chronic eczematous eruptions, fresh lesions of lupus erythematosus and scleroderma, pemphigus vulgaris, vitiligo, exfoliative dermatitis, scarlet fever, Buschke's scleredema adultorum, polyarteritis nodosa, poikiloderma atrophicans vasculare, myxedema (especially the circumscribed type), granuloma pyogenicum, and in all tumors, whether benign or malignant (2, 7, 18, 19, 20).

TABLE II

*Mast cell counts in 33 skin biopsy specimens of histologically normal skin*

| Age Group | Case No. | Mast Cells per Cubic Millimeter of Corium | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Neck | Hand | Forearm | Arm | Chest | Back | Abdomen | Thigh | Leg |
| 0–20 | 5 B | | | | 7,424 | | | | | |
| | 37 | | | | | | 2,048 | | | |
| 21–40 | 6 | | | | | 1,792 | | | | |
| | 10 B | | | | | 6,144 | | | | |
| | 28 | | | | 5,760 | | | | | |
| | 29 | | | 1,280 | | | | | | |
| | 44 | | | | | | | 6,144 | | |
| | 45 | | | | | | | | | 2,816 |
| | 49 | | 2,176 | | | | | | | |
| | 52 | | | 4,096 | | | | | | |
| | 55 | | | | | | | | 5,504 | |
| 41–60 | 24 | | | | | | | | 2,816 | |
| | 30 | | | | | | | | 8,192 | |
| | 31 | | | | | | | 8,704 | | |
| | 32 | | | | | | 10,112 | | | |
| | 33 | 6,912 | | | | | | | | |
| | 35 | | | | 8,576 | | | | | |
| | 40 | | | | | | | | | 1,536 |
| 41–60 | 46 | 3,968 | | | | | | | | |
| | 48 | | | | | 3,712 | | | | |
| | 50 | | | 2,560 | | | | | | |
| | 51 | | | | | | | | 6,016 | |
| | 54 | | | | | | 7,552 | | | |
| 61 up | 1 | | | | | | | 2,560 | | |
| | 2 | | | | | | | | 3,328 | |
| | 3 | | | | | | | | 10,112 | |
| | 38 | | | | | | | | 2,816 | |
| | 39 | | | 4,864 | | | | | | |
| | 41 | | | | 2,048 | | | | | |
| | 42 | | | | | | | | 2,688 | |
| | 43 | | | | | | 5,504 | | | |
| | 53 | | | | | | | 5,760 | | |

On reviewing the literature we could find few studies dealing with the quantitative distribution of mast cells in the human skin which were supported by numerical data.

Mills *et al* (21) made mast-cell counts on human skin obtained postmortem and fixed at intervals up to 24 hours after removal from the body. The number of mast cells per square centimeter of human dermis in 7 microns-thick sections in three subjects was as follows: 173 to 333; 3,658 to 5,860; and 3,307 to 5,632. They infer that mast cell counts done on refrigerated human tissues and fixed in formalin within 24 hours after death can be accepted in each case as representing the number of these cells present during life. Binazzi and Rampichini (22) undertook mast cell counts in 12 different skin areas of 14 subjects of both sexes, aged 10–60. In all, 29 biopsy specimens were studied, each skin area being represented by 2 or 3 specimens. They found that the mast cell counts varied from area to area, the highest average rate occurring in the scrotum (177 per square millimeter) and the lowest in the leg (46 per square millimeter).

Hellström and Holmgren (14), on the other hand, determined the number of mast cells per cubic millimeter in the skin from the abdomen, calf and back of the foot, as well as in specimens removed from different portions of the heart, in autopsy material from 40 human subjects ranging in age from birth to 83 years.

TABLE III

*Mast cell counts in 17 dermatological conditions in 28 patients*

| Case No. | Diagnosis | Biopsy site | Age | Race | Sex | Mast Cells per Cubic mm | Mast Cells per Cubic mm in Normal Skin |
|---|---|---|---|---|---|---|---|
| 26 | Urticaria pigmentosa | Abdomen | 1½ | W | M | 262,400 | |
| 65 | Urticaria pigmentosa | Arm | 1½ | W | M | 384,000 | |
| 27 | Urticaria pigmentosa | Back | 15 | W | M | 278,400 | |
| 57 | Urticaria pigmentosa | Thigh | 4 mo. | W | F | 289,280 | |
| 25 | Pretibial myxedema | Leg | 47 | W | M | 17,536 | |
| 34 | Paget's disease | Nipple | 48 | W | F | 18,048 | |
| 56 | Discoid Lupus eryth. | Scalp | 47 | C | F | 5,760 | |
| 35 | Psoriasis | Arm | 68 | C | F | 11,136 | Arm: 8,576 |
| 5A | Psoriasis | Arm | 8 | W | M | 7,040 | Arm: 7,424 |
| 15 | Psoriasis | Thigh | 12 | | M | 2,304 | |
| 16 | Psoriasis | Leg | 44 | W | F | 6,016 | |
| 7 | Atopic dermatitis | Forearm | 27 | C | F | 15,400 | Arm: 9,344 |
| 13 | Contact dermatitis | Abdomen | 22 | C | F | 6,528 | |
| 14 | Subacute eczematous dermatitis | Leg | 61 | C | M | 2,688 | |
| 10A | Seborrheic dermatitis | Chest | 36 | C | F | 3,840 | Chest: 6,144 |
| 23 | Seborrheic dermatitis | Back | 34 | C | F | 1,152 | |
| 11 | Mycosis fungoides | Back | 65 | C | F | 28,418 | |
| 59 | Mycosis fungoides | Back | 71 | W | M | 12,544 | |
| 19 | Sarcoidosis | Forearm | 34 | C | F | 768 | |
| 70 | Sarcoidosis | Neck | 30 | C | M | 1,792 | |
| 21 | Sarcoidosis | Forearm | 26 | C | M | 768 | |
| 22 | Keratosis senilis | Face | 46 | W | F | 5,888 | |
| 20 | Squamous cell carcinoma | Cheek | 83 | W | M | 7,680 | |
| 58 | Neurofibromatosis | Breast | 20 | C | F | 18,176 | |
| 8 | Erythroderma | Forearm | 61 | W | M | 19,968 | |
| 12 | Lichen simplex chronicus | Scalp | 78 | W | F | 10,496 | |
| 60 | Lichen planus (hypertrophic) | Forearm | 58 | W | F | 13,824 | |
| 69 | Basal cell epithelioma | Neck | 69 | W | F | 14,720 | |

Their sections were fixed in 10% formalin solution and stained with dilute solution of toluidine blue. They found that the mast cell population decreases with advancing age from about 7000 cells per cubic millimeter in infancy to about 1000 per cubic millimeter at the age of 70–80. The maximum figures, about 8500 cells per cubic millimeter, were found in the youngest cases while the lowest values, about 200, were observed in cases over 65 years. They also worked out the coefficient of regression of the mast cell rate with advancing age. According to their data, the rates for the age group used in the present study, 27–50 years, would fall in the range 1700–3000, or its whereabouts, for the skin of the abdomen, the calf and the dorsum of the foot. In subjects below the age of 40 years, the mast cell rate was higher for women than it was for men, but the difference was not significant statistically. However, the rate seemed to drop more rapidly with age in women than it did in men.

Simpson (23) concludes, after reviewing recent contributions to the knowledge of the mast cell and its fluctuations with age, that there is no common pattern of mast cell change with age, and that the mast cell population of any tissue is controlled largely by local factors, either physiological or pathological.

It may be noted that in the above-mentioned studies, apart from that of Binazzi and Rampichini (22), the mast cell counts were performed in autopsy material and included subjects suffering from systemic disease (14, 21). Furthermore, while some of these authors (14) expressed the rate per cubic millimeter,

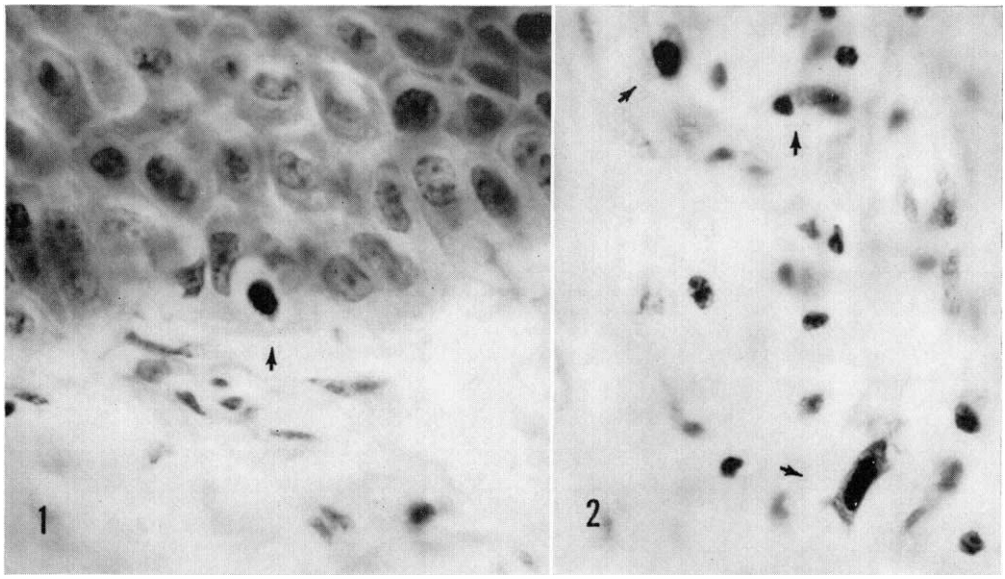


FIG. 1. A mast cell in the basal layer of the epidermis. The nucleus is small and is surrounded by a narrow zone of granular cytoplasm. Toluidine blue; × 990.
FIG. 2. Mast cells of various shapes in the corium. Toluidine blue; × 990.

others expressed it per square centimeter (21) or square millimeter (22). The figures obtained in the present study are much higher than those reported by Hellström and Holmgren (14). This variance is probably due to the different conditions under which the skin material was obtained, the method of tissue fixation and the staining technic employed in this study. We could not observe prominent variation with age because it was not feasible to obtain enough biopsy material from the younger age group in a reasonable period of time.

Although the data presented in this paper are far from being comprehensive as far as the mast cell population of the skin is concerned, an attempt has been made to establish a yardstick by which variations from the norm may be detected and thus may be of service in the diagnosis of certain pathological conditions. It is the intention of the authors to continue along the same lines and compile further data.

## SUMMARY

Mast cells were identified by their metachromatic staining reactions with toluidine blue in 95 biopsy specimens from normal and diseased Caucasian and Negro skin. The best staining results were obtained by fixing the tissue in absolute alcohol and treating it with dilute toluidine blue at pH 3.

In 34 preparations of skin taken from six different body areas in seven normal adults aged 27–50, a mean mast cell count of 7,225 per cubic millimeter was determined, but individual counts ranged from 5,120 and 9,472. No statistically significant variations could be found in our limited sample in the mast cell population in relation to age, race, and sex, or on their distribution according to regions of the body.

Mast cell counts were also undertaken in 33 normal skin specimens and 17 skin diseases (in 28 patients). The possible significance and value of the observations are discussed.

## REFERENCES

1. Mast Cells and Basophils. Ed. J. Padawer. Ann. N. Y. Acad. Sci. **103**(1): 1–492, 1963.
2. MICHELS, N. A.: The mast cells. In Handbook of Hematology, Ed. H. Downey, **1:** 232–372. New York, Hoeber, 1938.
3. RILEY, J. F.: The Mast Cells. London, England, E. S. Livingstone, 1959.
4. KELSALL, M. A. AND CRABB, E. D.: Lymphocytes and Mast Cells. Baltimore, Md., Williams & Wilkins Co., 1959.
5. MONTAGNA, W., CHASE, H. B. AND MELARAGNO, H. P.: Histology and cyto-chemistry of human skin. I. Metachromasia in the mons pubis. J. Nat. Cancer Inst., **12:** 591, 1951.
6. BLOOM, F.: Effect of cortisone on mast cell

tumors (mastocytoma) of the dog. Proc. Soc. Exp. Biol. Med., **79:** 651, 1952.
7. Asboe-Hansen, G.: Hormonal effects on connective tissues. In Transactions of the Fifth Conference on Connective Tissues, pp. 123–182. New York, Josiah Macy Foundation, 1954.
8. Wegelius, O. and Asboe-Hansen, G.: Hormonal effects on mast cells. Studies on living connective tissue in the hamster cheek pouch. Acta Endocr., **22:** 157, 1956.
9. Boseila, Abdel-Wahed A.: Hormonal influence on blood and tissue basophilic granulocytes. Ann. N. Y. Acad. Sci., **103**(1): 394, 1963.
10. Baer, R. L., Bersani, R. and Pelzig, A.: Effects of reserpine on urticaria pigmentosa. J. Invest. Derm., **32:** 5, 1959.
11. Bengt, H.: Release of histamine in urticaria pigmentosa. Lancet, **1:** 867, 1957.
12. Norton, S.: Quantitative determination of mast cell fragmentation by compound 48/80. Brit. J. Pharmacol., **9:** 494, 1954.
13. Brack, E.: Uber Bindegewebsmastzellen in menschlichen Organismus. Folia Haemat., **31:** 202, 1925.
14. Hellström, B. and Holmgren, H.: Numerical distribution of mast cells in the human skin and heart. Acta Anat., **10:** 81, 1950.
15. Williams, H.: Plasma-cells and mast-cells. A critical review of recent literature. Amer. J. Med. Sci., **119:** 702, 1900.
16. Sundburg, M.: On the mast cells in human vascular wall. A quantitative study on the changes at different ages. Acta Path. Microbiol. Scan., Suppl., **107:** 1, 1955.
17. Webb, R. L.: Peritoneal reactions in the white rat with special reference to the mast cells. Amer. J. Anat., **49:** 283, 1931.
18. Michels, N. A.: Medullary and non-medullary erythropoiesis with special reference to the plasma-cell erythrophage or Russell body cell, and to the erythrocatheretic (erythrolytic) function of lymph nodes and hemal nodes. Amer. J. Anat., **57:** 439, 1935.
19. Bierich, R.: Uber die Beteiligung des Bindegewebes bei der experimentellen Krebsbildung. Virchows Arch., **239:** 1, 1922.
20. Rupe, E. C.: Mast cells in non-neoplastic diseases of man and their relationship to the basophilic leucocyte. Ann. N. Y. Acad. Sci., **103**(1): 436, 1963.
21. Mills, J., Strickland, G. and Paterson, J. C.: The validity of tissue mast-cell counts in postmortem material. Arch. Path. (Chicago), **66:** 330, 1958.
22. Binazzi, M. and Rampichini, L.: 1959. Investigations on regional distribution of mast cells in human skin. Ital. Gen. Rev. Derm., **1:** 17, 1959.
23. Simpson, W. L.: Distribution of mast cells as a function of age and exposure to carcinogenic agents. Ann. N. Y. Acad. Sci., **103**(1): 4, 1963.